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# Processing seabuckthorn fruit for antioxidant rich jam development and shelf stability assessment

Meenakshisundaram Selvamuthukumaran* & Farhath Khanum

Biochemistry & Nutrition Division Defence Food Research Laboratory, Siddartha Nagar, Mysore – 570 011, Karnataka, India
E-mail: selkuma@hotmail.com

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Seabuckthorn [*Hippophae rhamnoides* L. syn. *Elaeagnus rhamnoides* (L.) A. Nelson] fruit was processed for development of antioxidant rich jam by employing response surface methodology as a statistical tool. The ingredients, viz. sugar and carrageenan were chosen as independent variables, while sensory attributes, i.e. taste and consistency as dependent variables. Effects of various independent variables on chosen response show that the ingredient sugar had more prominent effect on taste score and carrageenan on consistency score, respectively. The optimum conditions to yield maximum scores of taste and consistency of jam were sugar of 85 gm and carrageenan of 3.4 gm per 100 gm of recipe. Jam contains more natural antioxidants with good texture when compared to commercial products. Shelf stability of jam shows that significant changes in nutritional constituents were observed except acidity and *p*H during the entire storage period. The microbial population of stored sample was found to be non detectable and product showed a stability of 8-months at room temperature.

**Keywords:** Antioxidant rich jam, Shelf stability, Response surface methodology, Vitamin E, *Hippophae rhamnoides*, Carrageenan

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Seabuckthorn [*Hippophae rhamnoides* L. syn. *Elaeagnus rhamnoides* (L.) A. Nelson] is a deciduous spiny shrub or small tree between 2-4 m high, widely distributed throughout the temperate zone of Asia and Europe[1]. The plant is reported to have considerable medical value[2], being useful for the treatment of skin disorders resulting from bed confinement, stomach and duodenal ulcers, cardiovascular diseases and perhaps growth of some tumors. These beneficial effects have made seabuckthorn products desirable for medicinal and cosmetic purposes.

The plant is primarily valued for its golden-orange fruits which provide anthocyanins, carotenoids and other healthful components. They are also rich in nutrients such as carbohydrates, organic acids, amino acids, vitamin C, E and the vitamin content of seabuckthorn fruit is much higher than any other fruit or vegetable[3, 1, 4, 5, 6].The pulp derived from fresh berries are a good source of several minerals, phytochemicals, antioxidants and they are immensely flavored with exotic flavor and considered to be a good appetizer, which has health imparting attributes [7, 5, 8]. Seabuckthorn products based on fruit pulp or juices contain more phytochemicals and therefore they are considered to have therapeutic and chemo preventive benefits[2, 9].

Fresh seabuckthorn fruits are inherently more liable to deterioration under tropical conditions owing to high ambient temperatures and humidities, pest and disease infestations, poor handling and storage facilities. One attractive and effective means of fruit utilization and preservation is processing them into jam. It can be used as a bread spread and also as filler for many other bakery products, viz. cake, pastries and biscuits. The product is also liked by consumers of all the age groups, i.e. especially among children and they are having maximum portion of fruit than any other product.

The shelf stability of a product plays a crucial role in acceptance of the product after a prolonged storage period and it also helps in use of such product by consumers without any restriction. Therefore keeping all those aspects in view, the present study was undertaken to develop jam from wonderful fruit of seabuckthorn and to give a product of improved nutritional quality with high amount of carotenoids,

*Corresponding author

vitamin E and several other natural antioxidants and also to make them available for consumption throughout the year in processed form without any deterioration.

## Methodology

### Raw materials

Seabuckthorn berries (*H. rhamnoides* L. ) were brought from Field Research Laboratory (Leh, Himachal Pradesh, India) by airlifting and kept frozen at (-20˚C) until further studies. Food grade commercial sucrose and sodium bicarbonate were purchased from local market, while carrageenan was procured from Himedia Laboratories Limited (Mumbai, India).

### Experimental design

A central composite rotatable second order design was adopted to optimize the development of seabuckthorn jam. This includes a total of 14 experimental runs with 2 factorial points, 2 axial points and 1 centre point. The axial points represented the extremes, high and low values of the independent variables and were added to the factorial design to provide for estimation of curvature of the model[10]. Based on the preliminary trial formulations, the two independent variables affecting the quality of seabuckthorn jam were found to be: levels of sugar ($X_1$) and carrageenan ($X_2$). These coded variables $X_1$ and $X_2$ each at five different levels were: -1.414 (lowest level); -1 and 0 (middle levels); +1 and +1.414 (highest level). The levels used for each variable for seabuckthorn jam is given in Table 1. The data pertaining to each experiment were statistically analyzed using the PROCRSREG function of the Statistical Analysis System [11], and this software package was used to fit the second order polynomial models. The model proposed for each response was given below:

$$Y = \beta0 + \beta1X1 + \beta2X2 + \beta11\ X12 + \beta22\ X22 + \beta12\ X1X2 \quad (1)$$

Where $\beta_0$ is the value of the fitted response at the center point of the design; $\beta_1$ and $\beta_2$ are the linear regression terms; $\beta_{11}$ and $\beta_{22}$ are the quadratic regression terms; $\beta_{12}$ is the cross – product regression terms; $X_1$ and $X_2$ are the proportional level of two coded variables[12]. Response surfaces showing the effects of two chosen parameters on selected responses of seabuckthorn jam were made using a surfer program of Surfer Access System[13].

### Development of seabuckthorn jam using response surface methodology

The seabuckthorn jam was developed as per the standard procedure (Fig. 1) using RSM. A combination of ingredients, viz. sugar and carrageenan was used to obtain taste and consistency to individual treatment with a view to increase the levels of both sugar and carrageenan. The optimum level of single treatment was used as center value for the RSM experiments. The level of sugar ranged between 70.86 to 99.14 gm and carrageenan level was in the range of 2.55 to 3.54 gm /100 gm of jam recipe. The different variables and their levels, central composite design arrangement for preparation of seabuckthorn jam were given in Tables 1 & 2.

### Jam preparation

#### *Processing of seabuckthorn berries*

The frozen seabuckthorn berries were thawed to room temperature, sorted and washed to remove any adhering stalks and leaves. The cleaned berries were partially crushed in mixer grinder at lower speed, seeds were separated and removed manually from it and once again crushed well at higher speed to get coarse pulp. The fine pulp was obtained by filtering the seabuckthorn coarse pulp through the stainless steel sieve having screen size of 30-mesh. The fine pulp was pasteurized at 85˚C for 30 min in order to destroy the yeasts and moulds which could be present in berries, cooled and utilized for further product development as shown in Fig. 1.

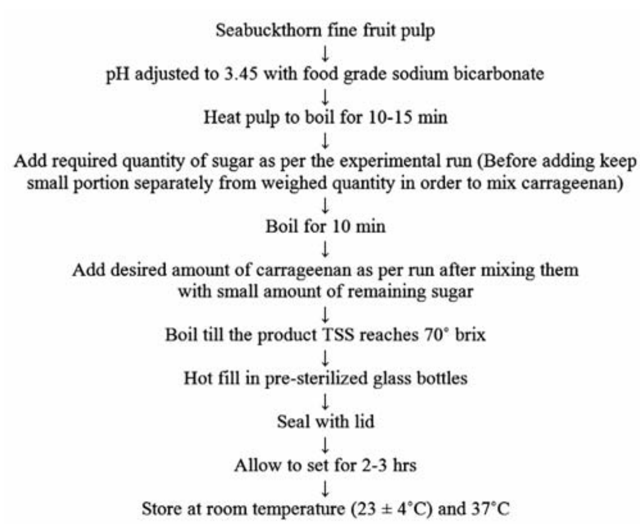


Fig. 1—Flow diagram for development of seabuckthorn jam

## Physico-chemical analysis

Proximate composition, viz. moisture, fat, protein, carbohydrate, dietary fibre and minerals were estimated as per the standard method[14]. Total soluble solids were determined using hand refractrometer (Erma, Tokyo, Japan). The titrable acidity was estimated as per the standard method[15]. The method of Lane and Eynon was followed for the determination of total and reducing sugar[15]. The 2, 6-dichlorophenol-indophenol titration method[15] was used for the estimation of ascorbic acid content. The total carotenoids were determined using an ultra violet – visible recording spectrophotometer at 450 nm (UV 1601, Shimadzu Corp., Columbia, USA) as per the standard method[16]. The total anthocyanins were estimated as per the procedure[15], spectrophotometrically at 535 nm. The total phenol content was measured with the Folin-Ciocalteu's reagent, spectrophotometrically as per the standard procedure [15].Vitamin E was estimated using Ferric Chloride-Dipyridyl, spectrophotometrically as per the standard method[17]. Antioxidant activity was estimated spectrophotometrically by DPPH method[18]. The degree of browning in the jam sample was estimated as per the standard method [15]. The texture analysis of jam was measured using a TAHDI Texture Analyzer (Stable Microsystems , Surrey, UK ) with 10 mm diameter perplex cylindrical probe. Texture Expert Software was used to analyze the data (Stable Microsystems, Surrey, UK).

## Sensory evaluation

A panel with 15 trained judges, aged 30-55 yrs, with sensory evaluation experience in fruit products, evaluated the different seabuckthorn jams. The coded (3 digit) samples were presented one at a time in random order to the judges. The judges were asked to evaluate for colour, taste, aroma, consistency and overall acceptability of the given products. A common Hedonic quality assurance scale with 9 for like extremely to 1 for dislike extremely was used for evaluation.

## Microbial analysis

The microbial load in the stored jam samples were analyzed initially and once in 60 days upto a period of 240 days. The total plate count (TPC), coliform, spores, yeasts and moulds were enumerated by serial dilution as per the standard method[15].

## Statistical analysis

All experiments were run in triplicate. Data were subjected to analysis of variance (ANOVA) using Microsoft Excel 2000 (Microsoft Corp., Washington, USA).

## Results and discussion

### Proximate composition and physico-chemical characteristics of seabuckthorn fruit and pulp

The colour of the seabuckthorn fruit was orange-yellow, oval shaped, 5-7 mm in size and has sour taste. The proximate composition results of fruit

Table 1—Variables and their levels for the central composite design of seabuckthorn jam

| | | | Coded and uncoded variable levels | | | | |
|---|---|---|---|---|---|---|---|
| Variables | Unit | Symbol | -1.414 | -1 | 0 | +1 | +1.414 |
| Sugar | g | $X_1$ | 70.86 | 75 | 85 | 95 | 99.14 |
| Carrageenan | g | $X_2$ | 2.55 | 2.70 | 3.05 | 3.40 | 3.54 |

Table 2—Central composite design arrangement for seabuckthorn jam

| Experiment number | Variable levels | | | |
|---|---|---|---|---|
| | Coded variables | | Uncoded variables* | |
| | $X_1$ | $X_2$ | Sugar (gm) | Carrageenan(gm) |
| 1 | -1 | -1 | 75 | 2.70 |
| 2 | +1 | -1 | 95 | 2.70 |
| 3 | -1 | +1 | 75 | 3.40 |
| 4 | +1 | +1 | 95 | 3.40 |
| 5 | -1.414 | 0 | 70.86 | 3.05 |
| 6 | +1.414 | 0 | 99.14 | 3.05 |
| 7 | 0 | -1.414 | 85 | 2.55 |
| 8 | 0 | +1.414 | 85 | 3.54 |
| 9 | 0 | 0 | 85 | 3.05 |
| 10 | 0 | 0 | 85 | 3.05 |
| 11 | 0 | 0 | 85 | 3.05 |
| 12 | 0 | 0 | 85 | 3.05 |
| 13 | 0 | 0 | 85 | 3.05 |
| 14 | 0 | 0 | 85 | 3.05 |

* For 100 gm of jam by keeping pulp as constant variable, i.e. 100 gm for all experimental runs.

shows that it has good amount of carbohydrate (29.08 %), fat (9.20 %), dietary fibre (2.24 %), protein (2.21%) and minerals viz. sodium (67.0 mg %), potassium (625.0 mg %), calcium (667.0 mg %), iron (17.0 mg %) and phosphorus (72.0 mg %). The physico-chemical characteristics of seabuckthorn fruit pulp reveals that the pulp had less amount of sugars (6.45 %), low *p*H (2.90) and high acidity (1.84 %). It also contained good amount of several antioxidants, viz. vitamin C (504.0 mg %), vitamin E (190.54 mg %), carotenoids (6.85 mg %), anthocyanins (1.48 mg %) and phenols (560.0 mg %) which finally led in higher antioxidant activity (89.23 %). Among the various antioxidants present, the concentration of total phenols, vitamin C and vitamin E was found to be higher followed by other antioxidants, viz. total carotenoids and total anthocyanins.

## Response results of seabuckthorn jam

The results pertaining to responses namely taste and consistency score of seabuckthorn jam are given in Table 3. The minimum and maximum values of these responses are highlighted in Table 3, which represents different set of conditions used for development of seabuckthorn jam. The score for taste varied from 5.86 - 8.45. The maximum score for taste of jam was of the sample prepared in experiment 8 and minimum in experiment 5. These experiments had the combinations of sugar 85 gm, carrageenan 3.54 gm /100 gm jam (Experiment 8) and 70.86 gm (sugar) and 3.05 gm (carrageenan) /100 gm jam (Experiment 5), respectively. The higher taste score might be due to use of optimum level of sugar during jam preparation, which might have brought desired sweetening effect to the final product.

The score for consistency of the jam in different experiments ranged from 5.09 - 8.62. The maximum and minimum values were exhibited in the samples of experiment 8 and 7 for combinations of sugar and carrageenan of 85, 3.54 gm and 85, 2.55 gm /100 gm of jam recipe, respectively. The higher consistency score may be due to use of carrageenan at an optimum concentration levels, which might have resulted in stronger gel structure due to degree of esterification, presence of optimum sugar concentration and also desired *p*H condition.

## Assessment on model of taste and consistency score for ingredients sugar and carrageenan

The second order polynomial equations were fitted to the experimental data, the equations (1.1- 1.2) were obtained and tested for their adequacy. The main results of this multiple regression were developed for dependent variable, with the corresponding coefficients of determination (Taste, $R^2$ = 0.82; Consistency, $R^2$ = 0.89). The model developed for taste and consistency score on ingredients, viz. sugar and carrageenan gave an indication as to how these variables should be related. The regression analysis showed that the experimental results were well described by the model within the experimental field ($p<0.05$).

Table 3—Experimental data*** of responses of dependent variables for seabuckthorn jam

| Experiment number | Dependent variables: Taste $Y_1$ | Consistency $Y_2$ |
|---|---|---|
| 1 | 6.06±0.25 | 5.32±0.40 |
| 2 | 7.50±0.27 | 5.12±0.21 |
| 3 | 6.33±0.24 | 8.42 ±0.26 |
| 4 | 7.78±0.33 | 7.75 ±0.18 |
| 5 | 5.86±0.32* | 8.08±0.28 |
| 6 | 8.05 ±0.40 | 5.26±0.17 |
| 7 | 7.18±0.27 | 5.09±0.16* |
| 8 | 8.45±0.29** | 8.62±0.19** |
| 9 | 7.37 ±0.15 | 6.35±0.17 |
| 10 | 7.33±0.20 | 6.32±0.22 |
| 11 | 7.41 ±0.11 | 6.41±0.26 |
| 12 | 7.32±0.21 | 6.33±0.24 |
| 13 | 7.30±0.21 | 6.36±0.39 |
| 14 | 7.38±0.15 | 6.33±0.37 |

*Minimum value of each dependent variable. **Maximum value of each dependent variables.

*** Mean ± standard deviation of triplicate analysis.

In case of the scores for taste the linear terms of sugar ($\beta_1$), carrageenan ($\beta_2$) and the quadratic terms of sugar ($\beta_{11}$) significantly ($p<0.05$) influenced the taste score of seabuckthorn jam. The equation obtained was:

$$Y1 = 7.35 + 0.75\ X1 + 0.29\ X2 - 0.31\ X12 \quad (1.1)$$

The equation of $Y_1$ i.e. taste score reveals that the ingredients, i.e. sugar and carrageenan were the factors influencing the taste score. Among those, the sugar effect was pronounced more and they specially influenced the taste score to a greater extent[19]. While, the later had only some influence on the chosen factors only.

In case of the scores for consistency the linear terms of sugar ($\beta_1$), carrageenan ($\beta_2$) and the quadratic terms of carrageenan ($\beta_{22}$) significantly ($p<0.05$) influenced the score for seabuckthorn jam. The equation obtained was:

$$Y2 = 6.35 - 0.61\ X1 + 1.34\ X2 + 0.22\ X22 \quad (1.2)$$

The equation of $Y_2$, i.e. consistency score reveals that likewise taste score both of the ingredients, viz. sugar and carrageenan influenced the consistency score. Among them, the carrageenan influenced the consistency score to a higher extent with significant effect compared to sugar ingredient alone.

**Effect of sugar and carrageenan on**

***a. Taste score of jam***

Fig. 2 A & B shows the 3-dimensional graph and contour plot for the effect of sugar and carrageenan on taste score of jam. The score increased with increase in amount of sugar as well as carrageenan. Sugar had more prominent effect than carrageenan. The sugar changes led to a sharp increase in the taste score of

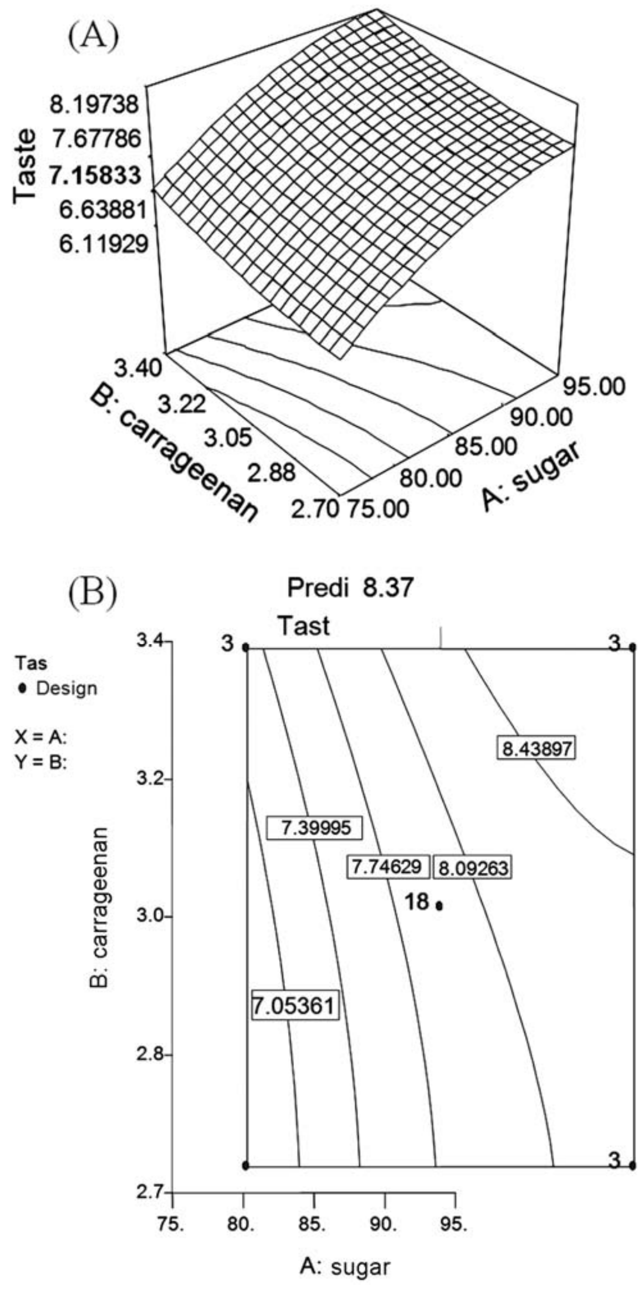


Fig. 2—Effect of sugar and carrageenan concentration on taste score of jam (A) and Contour plot (B) for taste score of jam as affected by sugar and carrageenan

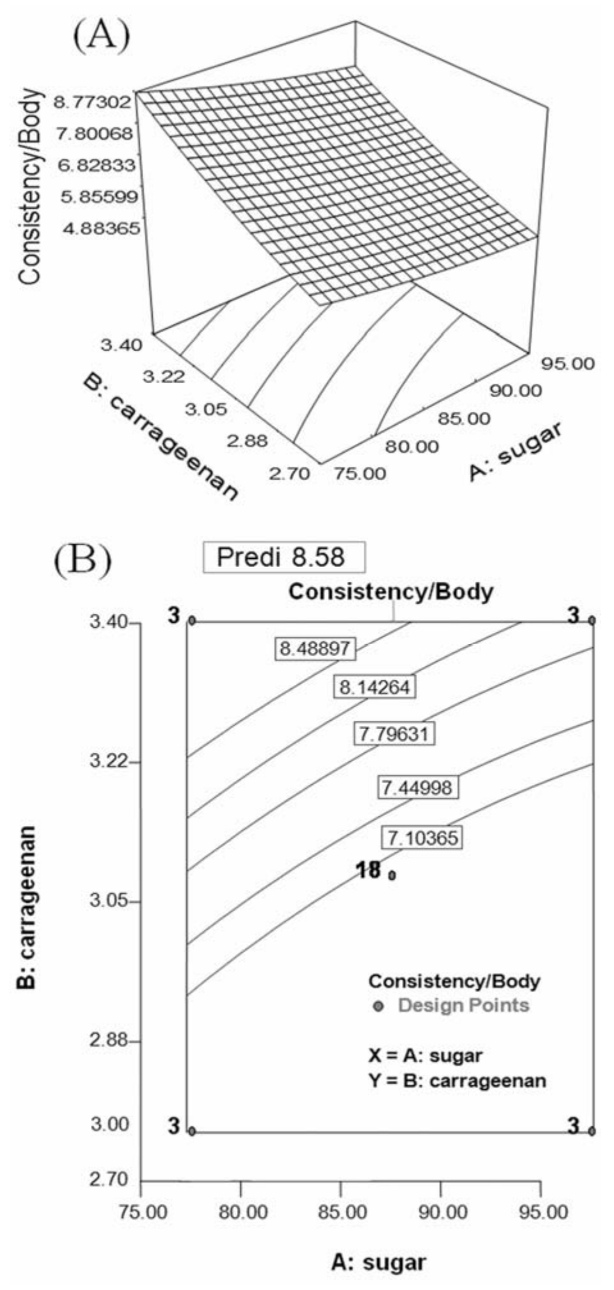


Fig. 3—Effect of sugar and carrageenan concentration on consistency score of jam (A) and Contour plot (B) for consistency score of jam as affected by sugar and carrageenan

the jam as compared to changes in carrageenan. Chompreeda *et al*.[19] also showed similar research findings of increase in taste score as a result of increase in concentration of sugar levels during preparation of chocolate flavored peanut beverage using RSM.

***b. Consistency score of jam***

Fig. 3 A & B shows the 3-dimensional graph and contour plot for the effect of sugar and carrageenan on consistency score of seabuckthorn jam. The increase in both sugar and carrageenan levels resulted in increase in consistency score of jam, however the effect of carrageenan was more than the effect of sugar levels. Dervisi *et al.*[20] were also reported similar results of achieving good and desirable texture in high hydrostatic pressure treated strawberry jam as a result of use of pectin concentration to an optimum extent.

## Optimization of jam

Numeric and graphic optimizations were carried out for the process parameters of the seabuckthorn fruit jam. The desired goals for each variable and response were chosen. The limit for each variable was narrowed down to obtain an optimal region. Each goal was chosen to be is: at target and is to maximize based on the sensory score of the developed product. Software has generated one optimum conditions of independent variables with the predicted values of responses, in the target of sugar= 85gm/100 gm and carrageenan concentration at maximum level=3.40 gm/100 gm for achieving the maximum scores of taste=8.37 and consistency=8.58. From the set of constraints and given outputs, contour plots of relevant and statistically significant responses were generated, and the overlaying of those plots is displayed in Fig. 4. The shaded area in Fig. 4 represents the $X_1$-$X_2$ domain satisfying the imposed criteria. The optimum processing conditions could be drawn from this shaded area and specific goals were achieved. For example, shaded area in Fig. 4 determines the following criteria and goals: sugar = 85 gm/100 gm, carrageenan=3.40 gm/m100 gm, taste score=8.37 and consistency score=8.58. The optimized jam was developed by using above ingredients for assessment of nutritional composition and shelf stability.

## Verification of second order polynomial model

The seabuckthorn fruit jam was prepared using the derived optimum formulation conditions to check the validity of the second order polynomial model. The

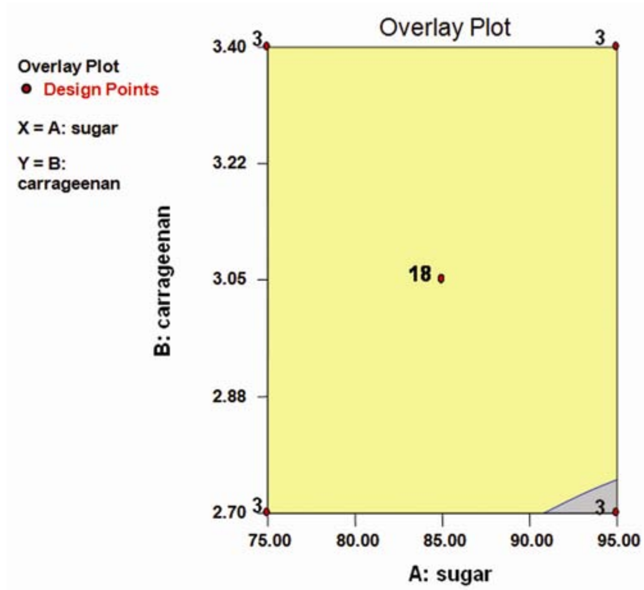


Fig. 4—Superimposed contour plot for significant responses as affected by sugar and carrageenan

actual values, i.e. experimental values for taste and consistency score were determined and compared with the predicted values of the second order polynomial model. The actual values of taste and consistency scores, i.e. 8.40 and 8.62 were found to be in close agreement with the predicted values of these scores, i.e. 8.37and 8.58 and they were also within the acceptable limits indicating the suitability of the model in predicting quality attributes of seabuckthorn fruit jam.

## Nutritional composition and antioxidant activity of jam

Nutritional composition and antioxidant activity of the seabuckthorn jam in comparison with other commercial jams is given in Table 4. The result showed that the seabuckthorn jam was significantly superior to the commercial jams in terms of nutritional value and antioxidant activity ($p<0.05$). The seabuckthorn jam exhibited more amount of antioxidants, viz. vitamins C, E, phenols, carotenoids and anthocyanins when compared to similar commercially available products. This may be due to presence of higher amount of natural antioxidants in raw material, i.e. seabuckthorn fruit, from which the product is being prepared. While the commercial fruit jam, i.e. pineapple and papaya jam showed very least above antioxidants, this may be due to presence of very less amount of these antioxidants in pineapple and papaya fruit, respectively. Vitamin E was found to be non detectable in both the commercial jams

Table 4—Nutritional composition and antioxidant activity** of optimized seabuckthorn jam in comparison with other commercial jams

| Composition | Seabuckthorn jam | Pineapple jam* | Papaya jam* |
|---|---|---|---|
| Total soluble solids (°brix) | 70.0 ± 0.0 | 70.0 ± 0.0 | 71.0 ± 0.0 |
| Acidity (%) | 0.80 ± 0.06 | 0.89 ± 0.04 | 0.82 ± 0.05 |
| Total sugars (%) | 65.8 ± 0.6 | 60.8 ± 0.7 | 61.3 ± 0.5 |
| Reducing sugars (%) | 21.6 ± 0.4 | 13.4 ± 0.6 | 15.8 ± 0.4 |
| Vitamin C (mg/100gm) | 175.1 ± 0.6 | 7.0 ± 0.5 | 5.4 ± 0.3 |
| Vitamin E (mg/100gm) | 76.2 ± 2.0 | ND | ND |
| Total carotenoids (mg/100gm) | 2.74 ± 0.72 | 0.12 ± 0.04 | 0.09 ± 0.03 |
| Total anthocyanins (mg/100gm) | 0.53 ±0.24 | 0.05 ± 0.01 | 0.03 ± 0.01 |
| Total phenols (mg/100gm) | 226.8 ± 2.0 | 23.2 ±1.6 | 18.0 ± 1.8 |
| Antioxidant activity (%) | 69.39 ± 0.61 | 8.16 ± 0.54 | 6.19 ± 0.47 |

*Commercial products. **Values are mean ± standard deviation of triplicate analysis. ND: not detectable.

except seabuckthorn jam. This may be due to absence of vitamin E content in these fruits, in which the jam is being developed. Chauhan *et al.*[3] also reported a similar results, i.e. more amount of carotenoids and vitamin C during comparison of a developed product, i.e. seabuckthorn jam with other fruit jams, viz. orange, apple and papaya fruit jams. The presence of antioxidants enriches the product with both nutritional and functional value. Therefore these products could be recommended as multibenefit vitamin-antioxidant rich nutritional foods for the consumers.

**Texture profile of jams**

Typical texturometer curves for a seabuckthorn jam and a commercial mango jam are shown in Figs. 5 A &B. The result shows that seabuckthorn jam exhibited more adhesiveness (0.313 NS), more cohesiveness (0.666), more gumminess (0.032) and more chewiness (0.031) when compared to commercial mango jam, i.e. 0.103 NS, 0.351, 0.019 and 0.019, respectively. The higher cohesiveness value corresponded to samples with a weak gel structure. The structure of a weak gel is mainly formed by temporary crosslink's which may be largely reformed after the applied stress is removed and hence it shows high cohesiveness[21]. In contrast, the mango jam showed low cohesiveness value. This may be because, when a strong gel is stressed beyond its elastic limit, permanent as well as temporary crosslink's are irreversibly destroyed, and the gel becomes broken, hence it shows low cohesiveness. Regarding for springiness, i.e. elasticity, only slight changes in values were noted for both the types. The values for gumminess and chewiness were high for seabuckthorn jam compared to mango jam, because they showed a semi solid rather than true solid behavior. The seabuckthorn jam exhibited less hardness value (0.048 N), this may be because of formation of weaker gels and non rigid nature of gels[21].

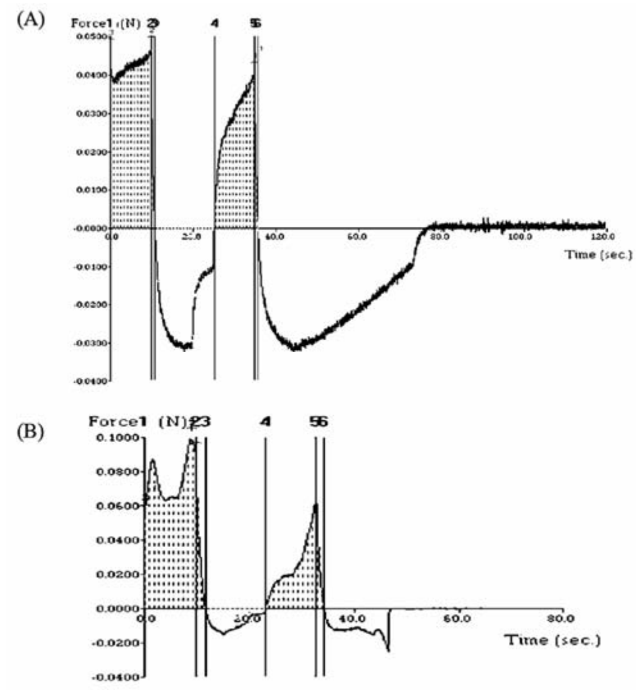


Fig. 5—Typical texturometer curve comparison of seabuckthorn jam (A) and commercial mango jam (B)

While the mango jam contains more hardness (0.055 N) and fracturability value (0.075 N). The texture parameters of seabuckthorn jam was found to be well comparable with commercial product. Raphaelides *et al.*[21] also observed a similar result of higher cohesiveness value, gumminess, chewiness and springiness value while studying the textural parameters of the developed peach jam after preparing with the effect of various blended sugars viz., sucrose, maltose and iso glucose.

## Storage stability of jam

The seabuckthorn jam immediately after its preparation was hot filled into presterilized glass bottles, sealed and stored at room temperature (23 ± 4˚C) and 37˚C.

## Physico – chemical evaluation of stored jam

### *Total soluble solids*

The initial total soluble solids (TSS) of the seabuckthorn jam were 70.0˚ brix and it increased to 73.3˚ and 73.9˚ brix, after 8 months of storage at room temperature (23 ± 4˚C) and at 37˚C, respectively (Table 5). The increase in TSS could be due to the conversion of polysaccharides into simple sugars during storage[22]. The increase was significant at the level of ($P < 0.05$).

### *Acidity and pH*

Acidity helps in lowering the *p*H and thereby increasing the strength of the jam. The initial jam acidity of 0.80% slightly decreased to 0.77 and 0.75%, after 8 months of storage at room temperature (23 ± 4˚C) and 37˚C, respectively (Table 5). The initial pH of the jam was 3.31 and this slightly increased to 3.34 and 3.36 after 8 months of storage at room temperature (23 ± 4˚C) and at 37˚C, respectively (Table 5). The changes in acidity and *p*H was found to be non significant ($P > 0.05$) at both the temperature of storage.

### *Total and reducing sugars*

The higher the sugar concentration, the greater is the jam strength. The initial total sugar content of the jam was 65.8% and it increased to 68.5 and 69.4% after 8 months of storage at room temperature (23 ± 4˚C) and at 37˚C, respectively (Table 5). This might be due to the partial hydrolysis of complex carbohydrates which could contribute towards increase in the total sugars in the stored product. The reducing sugar content of the seabuckthorn jam increased from 21.6% to 26.8 and 27.6% after 8 months of storage at room temperature (23 ± 4˚C) and at 37˚C, respectively (Table 5). The increase in reducing sugars might be due to hydrolysis or inversion of non-reducing sugars to reducing sugars[23]. The increase in total and reducing sugar content of the stored samples was significant at level of ($P<0.05$).

### *Vitamins C, E, total carotenoids, anthocyanins and phenols*

Vitamin C is one of the important vitamins and it is required for the growth and maintenance of good health. It is also a highly potent natural antioxidant. The initial vitamin C content of the jam was 175.1 mg/100 gm, but significantly decreased ($P<0.05$) to 77.0 and 15.7 mg/100 gm after 8 months of storage at room temperature (23 ± 4˚C) and at 37˚C, respectively (Table 5). This could be due to oxidation or degradation of the thermolabile ascorbic acid into dehydroascorbic acid upon storage. The vitamin C loss accounted to 56% at room temperature after 8 months of storage period, however when the storage temperature increased from room temperature to 37˚C the loss also further increased to 91% after 8 months of storage period.

Vitamin E is an important dietary constituent, as it is essential in maintaining the stability and integrity of the cell membrane. This is an antioxidant and free radical scavenger and its presence has been linked to prevention of chronic diseases and premature ageing, cancer, cardiovascular diseases and strokes [24]. The initial vitamin E content of seabuckthorn jam was 76.2 mg/100 gm, which significantly decreased ($P<0.05$) to 49.5 and 41.1 mg/100 gm, after 8 months of storage at room temperature (23 ± 4˚C) and 37˚C, respectively (Table 5). This might be due to its degradation, susceptibility to lipid auto oxidation, sensitive to oxidation and heat might have contributed to the depletion of vitamin E content in stored product at above temperatures. The loss of vitamin E accounts for 35% and 46% at room temperature and at 37˚C, respectively after 8 months of storage.

Carotenoids are important food constituents, imparting colour and nutritional value as pro-vitamin A apart from being strong antioxidants. The total carotenoids content of jam 2.74 mg/100 gm initially decreased significantly ($P<0.05$) to 1.37 and 0.82 mg/100 gm, after 8 months of storage at room temperature (23 ± 4˚C) and 37˚C, respectively (Table 5). This might be due to oxidation/degradation at these temperatures. The loss in total carotenoids accounted for 50% under room and 70% at 37˚C temperature condition after 8 months of storage.

Anthocyanins are responsible for the attractive colour of the product and they have been recognized as important antioxidants[25]. The initial total anthocyanin content of seabuckthorn jam 0.53 mg/100 gm decreased

Table 5—Physico chemical analysis* of stored seabuckthorn jam (n=3)

| Storage Period in months | TSS (˚brix) | | Total acidity (%) | | *p*H | | Total sugars (%) | | Reducing sugars (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 23 ± 4˚C | 37˚C | 23 ± 4˚C | 37˚C | 23 ± 4˚C | 37˚C | 23 ± 4˚C | 37˚C | 23 ± 4˚C | 37˚C |
| 0 | 70.0 ± 0.3 | 70.0 ± 0.3 | 0.80 ± 0.02 | 0.80 ± 0.02 | 3.31 ± 0.04 | 3.31 ± 0.04 | 65.8 ± 0.6 | 65.8 ± 0.6 | 21.6 ± 0.4 | 21.6 ± 0.4 |
| 1 | 70.6 ± 0.2 | 70.8 ± 0.1 | 0.80 ± 0.02 | 0.80 ± 0.02 | 3.31 ± 0.04 | 3.31 ± 0.04 | 66.2 ± 0.4 | 66.7 ± 0.3 | 21.9 ± 0.3 | 22.6 ± 0.3 |
| 2 | 70.8 ± 0.1 | 71.6 ± 0.4 | 0.80 ± 0.02 | 0.79 ± 0.01 | 3.31 ± 0.04 | 3.32 ± 0.03 | 66.8 ± 0.5 | 67.0 ± 0.4 | 22.0 ± 0.5 | 23.5 ± 0.2 |
| 3 | 71.4 ± 0.4 | 71.8 ± 0.4 | 0.79 ± 0.01 | 0.78 ± 0.03 | 3.31 ± 0.04 | 3.32 ± 0.03 | 67.4 ± 0.4 | 67.8 ± 0.6 | 22.6 ± 0.4 | 24.6 ± 0.1 |
| 4 | 71.9 ± 0.5 | 72.4 ± 0.3 | 0.79 ± 0.01 | 0.78 ± 0.03 | 3.32 ± 0.03 | 3.33 ± 0.02 | 67.8 ± 0.2 | 68.3 ± 0.2 | 23.5 ± 0.2 | 25.4 ± 0.4 |
| 5 | 72.2 ± 0.2 | 72.8 ± 0.4 | 0.79 ± 0.01 | 0.77 ± 0.04 | 3.32 ± 0.03 | 3.34 ± 0.04 | 68.0 ± 0.1 | 68.6 ± 0.3 | 24.3 ± 0.6 | 26.3 ± 0.2 |
| 6 | 72.6 ± 0.3 | 73.2 ± 0.2 | 0.78 ± 0.03 | 0.76 ± 0.02 | 3.33 ± 0.02 | 3.35 ± 0.01 | 68.2 ± 0.5 | 68.9 ± 0.5 | 25.4 ± 0.7 | 26.6 ± 0.2 |
| 7 | 73.0 ± 0.1 | 73.6 ± 0.1 | 0.78 ± 0.03 | 0.76 ± 0.02 | 3.33 ± 0.02 | 3.35 ± 0.01 | 68.4 ± 0.6 | 69.0 ± 0.7 | 26.0 ± 0.5 | 27.0 ± 0.3 |
| 8 | 73.3 ± 0.3 | 73.9 ± 0.3 | 0.77 ± 0.04 | 0.75 ± 0.01 | 3.34 ± 0.04 | 3.36 ± 0.02 | 68.5 ± 0.4 | 69.4 ± 0.6 | 26.8 ± 0.7 | 27.6 ± 0.6 |

| Storage Period in months | Vitamin C (mg/100gm) | | Vitamin E (mg/100gm) | | Total carotenoids (mg/100gm) | | Total anthocyanins (mg/100gm) | | Total phenols (mg/100gm) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 23 ± 4˚C | 37˚C | 23 ± 4˚C | 37˚C | 23 ± 4˚C | 37˚C | 23 ± 4˚C | 37˚C | 23 ± 4˚C | 37˚C |
| 0 | 175.1 ± 0.3 | 175.1 ± 0.3 | 76.2 ± 1.2 | 76.2 ± 1.2 | 2.74 ± 0.81 | 2.74 ± 0.81 | 0.53 ± 0.21 | 0.53 ± 0.21 | 226.8 ± 1.7 | 226.8 ± 1.7 |
| 1 | 168.6 ± 0.2 | 150.8 ± 0.1 | 70.4 ± 1.7 | 69.8 ± 1.0 | 2.51 ± 0.68 | 2.14 ± 0.55 | 0.48 ± 0.32 | 0.44 ± 0.12 | 220.6 ± 1.4 | 197.5 ± 1.1 |
| 2 | 150.8 ± 0.1 | 112.6 ± 0.4 | 68.2 ± 1.9 | 67.4 ± 1.7 | 2.30 ± 0.92 | 1.87 ± 0.72 | 0.42 ± 0.11 | 0.40 ± 0.10 | 200.5 ± 1.3 | 176.8 ± 1.0 |
| 3 | 142.4 ± 0.4 | 91.8 ± 0.8 | 64.4 ± 1.0 | 62.5 ± 1.9 | 2.00 ± 0.79 | 1.57 ± 0.63 | 0.39 ± 0.21 | 0.32 ± 0.11 | 186.8 ± 1.2 | 142.3 ± 1.3 |
| 4 | 131.9 ± 0.5 | 70.4 ± 0.9 | 60.1 ± 1.1 | 58.4 ± 1.5 | 1.96 ± 0.88 | 1.29 ± 0.52 | 0.36 ± 0.10 | 0.27 ± 0.16 | 179.5 ± 1.1 | 116.9 ± 1.2 |
| 5 | 110.2 ± 0.2 | 58.8 ± 0.4 | 56.4 ± 1.5 | 51.7 ± 1.4 | 1.74 ± 0.45 | 1.09 ± 0.49 | 0.34 ± 0.23 | 0.21 ± 0.10 | 166.8 ± 1.2 | 90.7 ± 1.6 |
| 6 | 98.6 ± 0.6 | 43.2 ± 0.5 | 52.7 ± 1.4 | 47.4 ± 1.2 | 1.57 ± 0.63 | 0.98 ± 0.50 | 0.32 ± 0.21 | 0.17 ± 0.11 | 158.9 ± 1.0 | 81.9 ± 1.5 |
| 7 | 85.3 ± 0.7 | 28.6 ± 0.6 | 51.6 ± 1.3 | 43.6 ± 1.8 | 1.45 ± 0.51 | 0.90 ± 0.39 | 0.31 ± 0.16 | 0.14 ± 0.10 | 150.2 ± 1.5 | 75.8 ± 1.4 |
| 8 | 77.0 ± 0.3 | 15.7 ± 0.3 | 49.5 ± 1.6 | 41.1 ± 1.3 | 1.37 ± 0.70 | 0.82 ± 0.40 | 0.29 ± 0.17 | 0.10 ± 0.10 | 145.1 ± 1.2 | 70.3 ± 1.6 |

| Storage period in months | Browning (OD) | |
|---|---|---|
| | 23 ± 4˚C | 37˚C |
| 0 | 0.286 ± 0.03 | 0.286 ± 0.03 |
| 1 | 0.314 ± 0.06 | 0.402 ± 0.06 |
| 2 | 0.349 ± 0.07 | 0.496 ± 0.04 |
| 3 | 0.386 ± 0.09 | 0.621 ± 0.07 |
| 4 | 0.405 ± 0.05 | 0.789 ± 0.09 |
| 5 | 0.429 ± 0.03 | 0.845 ± 0.06 |
| 6 | 0.450 ± 0.02 | 0.907 ± 0.05 |
| 7 | 0.471 ± 0.01 | 1.004 ± 0.03 |
| 8 | 0.490 ± 0.03 | 1.139 ± 0.02 |

*Values are Mean ± SD.

significantly (P<0.05) to 0.29 and 0.10 mg/100 gm, after 8 months of storage under room temperature (23 ± 4˚C) and 37˚C, respectively (Table 5). This might be due to hydrolysis of protective 3-glucoside linkages to give unstable anthocyanins under these storage conditions[26]. The loss in total anthocyanins accounted for 44.5% and 81% under room and at 37˚C temperature conditions respectively, after 8 months of storage period.

The phenolic compounds contribute immensely towards better human health due to their multiple biological effects such as antioxidant capacity, antimutagenic and anticarcinogenic activity and antiinflammatory action[27, 28, 29]. The content of total phenol of the seabuckthorn jam was estimated to be 226.8 mg/100 gm initially and this was found to decrease significantly (P<0.05) to 145.1 and 70.3 mg/100 gm, after 8 months of storage at room temperature (23 ± 4˚C) and 37˚C, respectively (Table 5). This might be due to the sensitivity of the phenolic components to oxidation at above stored conditions. This data indicates that a considerable loss in total phenols occurred during the storage period. The loss in total phenols accounted for 36% and 69% under room temperature and at 37˚C, respectively, after 8 months of storage.

### *Browning*

Colour intensity is a measure of degree of browning which increased significantly (P<0.05) from 0.286 OD to 0.490 and 1.139 OD, after 8 months of storage at room temperature (23 ± 4˚C) and at 37˚C, respectively (Table 5). The increase in optical density indicated browning with advancement of storage period, which could be ascribed to oxidation and development of non-enzymatic browning (an amino acid sugar interaction) resulting in the formation of the dark pigment.

## Sensory evaluation of stored jam

The sensory scores of seabuckthorn jam samples stored at room temperature (23 ± 4˚C) and at 37˚C are presented in Table 6. Initially the seabuckthorn jam had an overall acceptability score of 8.3, on a nine-point Hedonic scale; score of 6.1, 'liked slightly' was taken as the limit of shelf life in the storage studies. The jam remained acceptable upto 8 months of storage at room temperature. However colour, aroma, taste, consistency and overall acceptability were found to reduce after 2 months of storage at 37˚C. This might be due to chemical reactions which leads to the formation of brown pigments which might have decreased the colour score[30], loss of volatile aromatic substances during storage period under these conditions might have decreased the aroma and taste score[31]. The decrease in colour and consistency score during storage might be due to copolymerization, interaction between phenolics and proteins as well as the formation of cation complexes with pectins during storage[32]. All these changes could collectively decrease the overall acceptability of the stored products at above conditions. Changes in the seabuckthorn jam had a significant effect (P<0.05) on colour, aroma, taste, consistency and overall acceptability during storage period for 8 months.

## Microbial evaluation of stored jam

The microbial population, viz. total bacteria, yeast and mould, coliform and spores of fresh and stored jam at room temperature (23 ± 4° C) and at 37˚C for a

Table 6—Sensory evaluation* of stored seabuckthorn jam (n=15)

| Storage Period in months | Colour | | Aroma | | Taste | | Consistency | Overall acceptability | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 23 ± 4˚C | 37˚C | 23 ± 4˚C | 37˚C | 23 ± 4˚C | 37˚C | 23 ± 4˚C | 37˚C | 23 ± 4˚C | 37˚C |
| 0 | 8.5 ± 0.3 | 8.5 ± 0.3 | 8.0 ± 0.6 | 8.0 ± 0.6 | 8.4 ± 0.4 | 8.4 ± 0.4 | 8.6 ± 0.6 | 8.6 ± 0.6 | 8.3 ± 0.4 | 8.3 ± 0.4 |
| 1 | 8.3 ± 0.7 | 7.3 ± 0.4 | 7.8 ± 0.4 | 7.1 ± 0.7 | 8.2 ± 0.3 | 7.3± 0.5 | 8.2 ± 0.4 | 7.4 ± 0.5 | 8.1 ± 0.9 | 7.2 ± 0.6 |
| 2 | 8.0 ± 0.8 | 6.5 ± 0.3 | 7.6 ± 0.5 | 6.2 ± 0.6 | 8.0 ± 0.4 | 6.2 ± 0.3 | 7.8 ± 0.6 | 6.5 ± 0.7 | 7.8 ± 0.5 | 6.3 ± 0.7 |
| 3 | 7.9 ± 0.4 | 5.2 ± 0.4 | 7.3 ± 0.4 | 5.6 ± 0.2 | 7.8 ± 0.5 | 5.7 ± 0.7 | 7.6 ± 0.5 | 5.8 ± 0.8 | 7.6 ± 0.8 | 5.5 ± 0.5 |
| 4 | 7.6 ± 0.6 | 4.9 ± 0.5 | 7.1 ± 0.7 | 5.3 ± 0.6 | 7.6 ± 0.6 | 5.4 ± 0.4 | 7.3 ± 0.4 | 5.3 ± 0.5 | 7.4 ± 0.6 | 5.2± 0.9 |
| 5 | 7.3 ± 0.4 | 4.5 ± 0.7 | 6.8 ± 0.3 | 5.0 ± 0.3 | 7.3 ± 0.5 | 5.1 ± 0.6 | 7.1 ± 0.3 | 5.0 ± 0.7 | 7.1 ± 0.4 | 4.9 ± 0.7 |
| 6 | 7.0 ± 0.5 | 4.3 ± 0.5 | 6.4 ± 0.7 | 4.8 ± 0.4 | 7.0 ± 0.4 | 4.8 ± 0.7 | 6.8 ± 0.9 | 4.8 ± 0.6 | 6.8 ± 0.6 | 4.6 ± 0.4 |
| 7 | 6.8 ± 0.4 | 3.9 ± 0.8 | 6.2 ± 0.6 | 4.5 ± 0.2 | 6.7 ± 0.8 | 4.6 ± 0.9 | 6.5 ± 0.7 | 4.5 ± 0.5 | 6.5 ± 0.5 | 4.3 ± 0.6 |
| 8 | 6.2 ± 0.3 | 3.7 ± 0.6 | 6.0 ± 0.5 | 4.2 ± 0.5 | 6.2 ± 0.3 | 4.2 ± 0.8 | 6.2 ± 0.8 | 4.2 ± 0.9 | 6.1 ± 0.7 | 4.0 ± 0.8 |

*Values are mean ± standard deviation.

period of 8 months was found to be non detectable. The absence of microbial population in the product directly reflects the hygiene during handling and storage. The results further reveal that the product is safe for consumption.

## Significance of study

Fresh seabuckthorn fruits are inherently more liable to deterioration under tropical conditions owing to high ambient temperatures and humidities, pest and disease infestations, poor handling and storage facilities. Therefore this study will find a way to effectively convert the fruit into jam by means of value addition. The resultant product can be used as a bread spread and also as filler for several bakery products. This type of product will be liked by consumers of all the age groups, i.e. especially among children and one significance of this product will be, they are having maximum portion of fruit than any other product. The entrepreneurs can also launch this product commercially by providing both nutritious and health oriented product to the consumers throughout the year without any deterioration.

## Conclusion

The studies indicated that the antioxidant rich jam can be developed naturally from wonderful fruit of seabuckthorn. This product can be utilized throughout the year especially during the off season period because of its processed form by means of value addition and they are much superior when compared to commercial jams as far as the contents of vitamin C, E, carotenoids, anthocyanins, total phenols and antioxidant activity. Therefore, this type of product can be recommended as an antioxidant booster for the consumers in health point of view. The product can be safely consumed upto a period of 8-months without any deterioration in its quality at room temperature condition.

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